Supporting Information

Coacervation of α-elastin studied by ultrafast nonlinear infrared spectroscopy

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Sample preparation

α-Elastin was prepared by acid hydrolysis[1] as follows:

i. Porcine aorta was dissected free of adhering fats and cut into rings of approximately 10 mm in length, avoiding any intercostal branches.

ii. The tissue was then placed in a beaker with ≥ 10 volumes of 0.1 M NaOH and heated in a water bath at 95˚C for 45 minutes.

iii. The insoluble residue was then transferred to a large volume of distilled water and maintained at 4˚C, whilst repeatedly changing the water until pH 7.0 was obtained.

iv. Following this, the samples were dried in an oven at 60˚C until completely dry and ground as finely as possible using a pestle and mortar.

v. The ground-purified elastin was then placed into a round-bottomed flask, with attached air condenser, containing approximately 8 volumes of 0.25 M oxalic acid.

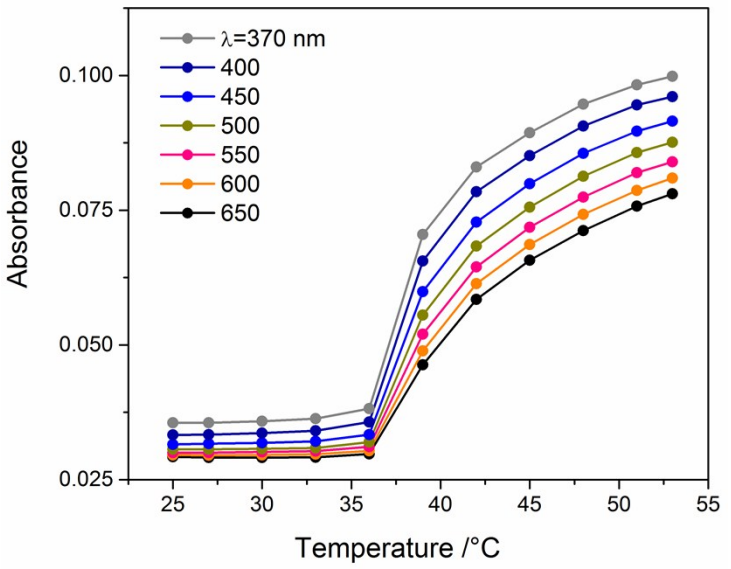
vi. The mixture was then heated on a steam bath at 100˚C for 1 hour before rapidly cooling centrifuging at 3000 rpm for 10 minutes.

vii. The supernatant was then carefully poured off and set aside whilst the insoluble residue was re-suspended in 0.25 M oxalic acid and washed in the centrifuge. The solution after washing was added to the supernatant from the previous spin and set aside. This cycle of heating and centrifugation was repeated a further 4-5 times until all the elastin powder had completely dissolved to leave a clear yellow solution.

viii. The solubilized elastin was then dialyzed in tubing of molecular cut off of 12-14000 Daltons (Medicell International Ltd, size 2) against distilled water at 4˚C until free of oxalate at pH 7.0 to remove the low molecular weight β-elastin fraction.

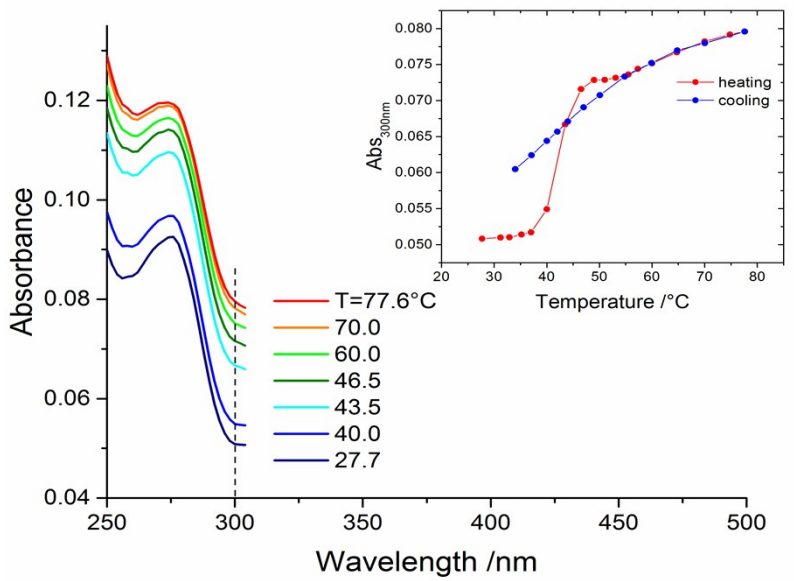
ix. The remaining product was then concentrated by osmosis in the dialysis tubing by the addition of a thin layer of polyethylene glycol (PEG, average molecular weight of 20 kDa), transferred into vials and stored at –20˚C until required.

Figure SI-1



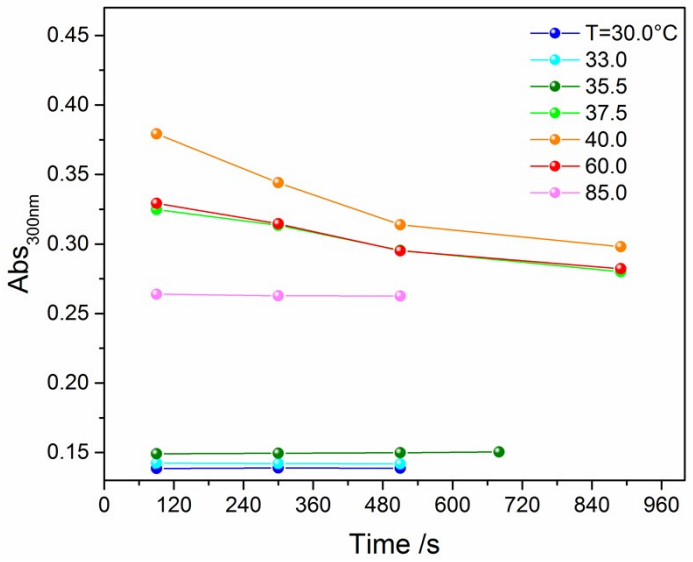
Plot of the UV-visible absorbance of α-elastin in PBS-D_2O at different wavelengths, in the range 370 to 650 nm, versus temperature for heating series.

Figure SI-2



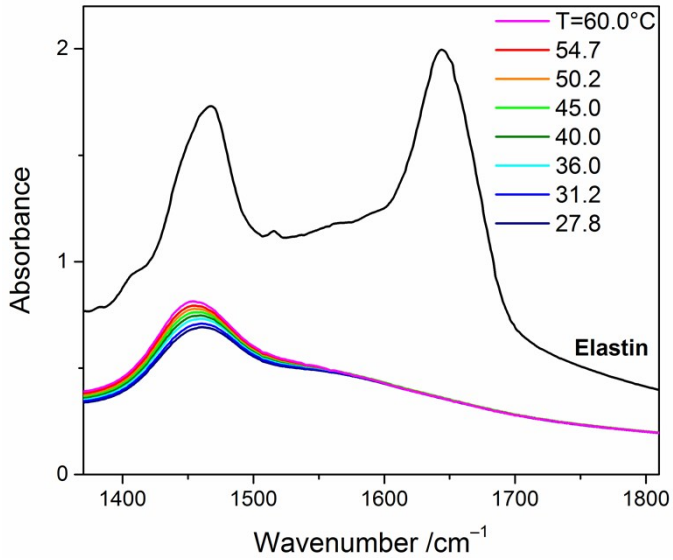
Evolution of the UV-visible absorption spectrum of α-elastin in PBS-D_2O as a function of temperature, in the range 27.7 to 77.6°C. The sample was not allow to thermalize at each temperature; heating was performed at a rate of 0.96°C/min. Band at 276 nm: tyrosine. Turbidity was assayed through light scattering at 300 nm (dashed line). Inset: plot of the absorbance at 300 nm versus temperature for both heating (red symbols) and cooling series (blue symbols).

Figure SI-3



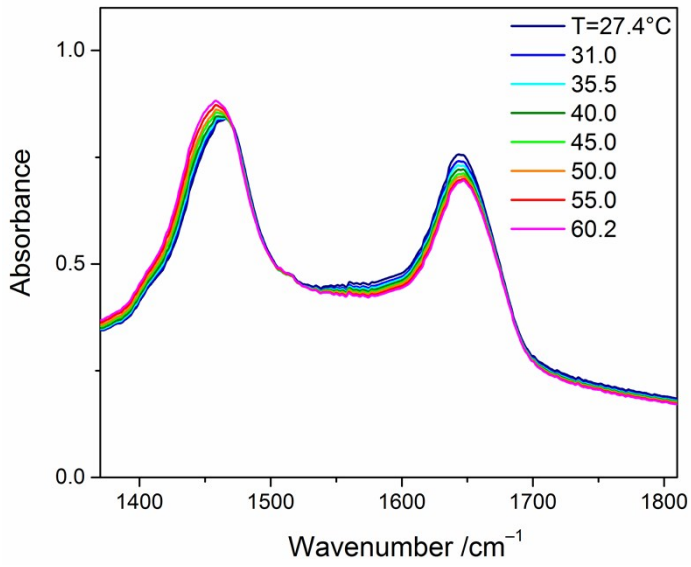
Plot of the UV-visible absorbance at 300 nm of α-elastin in PBS-D_2O versus time, up to 15 minutes after the start of the experiment at each temperature in the range 30 to 85°C. Coacervation kinetics are observed at 37.5, 40 and 60°C, while no changes in light scattering intensity are probed below 37.5°C and above 60°C. Constant values for the sample at 85°C, higher than those at the beginning of the experiment, are in line with the data in Figure 2 which indicate the formation of a stable coacervate.

Figure SI-4



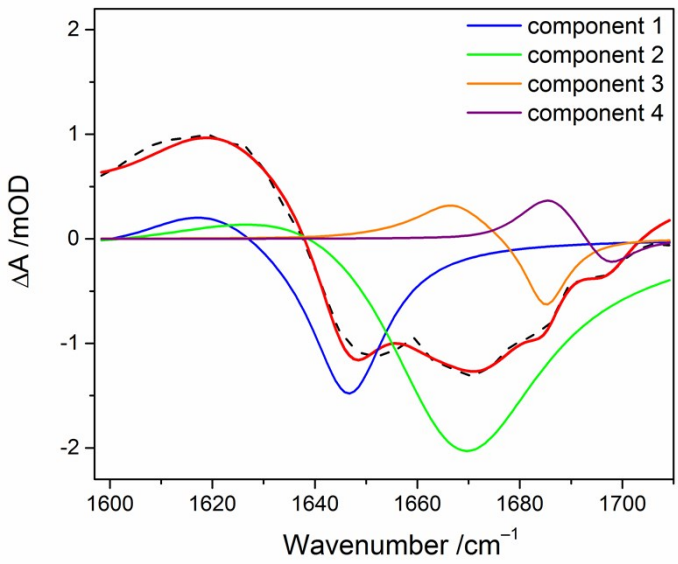
Evolution of the FTIR spectrum of a PBS-D_2O solution as a function of temperature, in the range 27.8–60 °C. Black line: spectrum of α-elastin in PBS-D_2O at 27°C. No change in intensity of the PBS-D_2O spectrum is observed in the region of the amide I band, 1600–1700 cm^{-1}.

Figure SI-5



Evolution of the FTIR spectrum of α-elastin in D_2O as a function of temperature, in the range 27.4–60.2 °C. In the absence of PBS, only a small change in the amide I band shape is observed.

Figure SI-6



Dashed line: Broadband nonlinear IR spectrum of α-elastin in PBS-D_2O measured at 0.1 ps time delay. Red line: Fitting curve resulting from a superposition of four components, each having a positive part (ESA) and a negative part (bleaching and stimulated emission). Blue, green, orange and violet lines: components of the fit using Voigt functions; parameters are listed in Table SI-1.

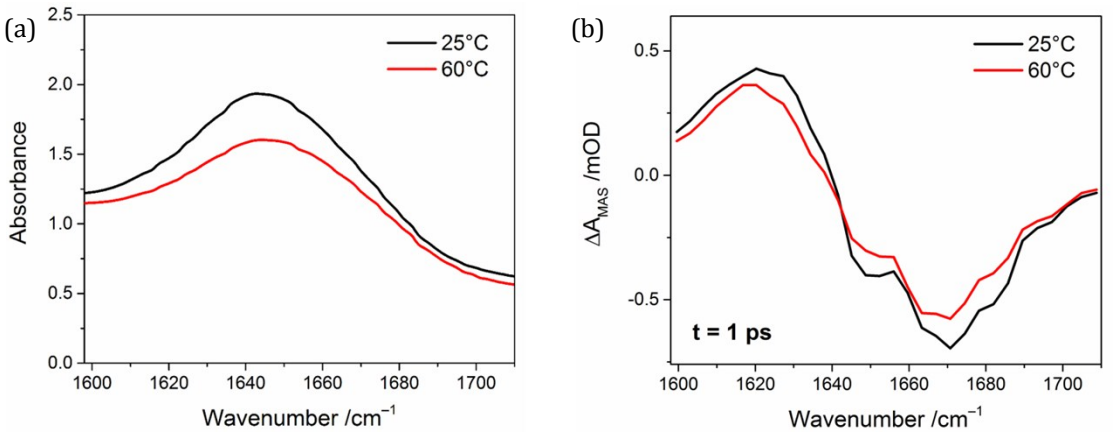
Fit results in Figure SI-6 show that the broadband transient amide I profile is a superposition of (at least) four components, each contributing a local minimum to the negative part of the signal. Each component has its own transient profile with intensity *I* (difference in Δ*A* between the maximum and the minimum of the transient signal) and anharmonicity Δ (frequency separation between the maximum and the minimum) listed in Table SI-1. A high spectrally resolved broadband IR spectrum such as that shown in Figure SI-6 is only indicative of the minimum number of possible components; no information on real frequency position and (in-)homogeneous broadening of the components can be inferred.

Table SI-1. Parameters[a] of the Exponential Fit Applied to the Broadband Transient of a α-Elastin Solution at 0.1 ps Time Delay.

Component	*I* /mOD	Δ /cm^{-1}
1	1.68	28
2	2.16	41
3	0.94	18
4	0.59	15

[a] Values are reported as *I*, difference in ΔA between the maximum and minimum of the transient signal; Δ, anharmonicity.

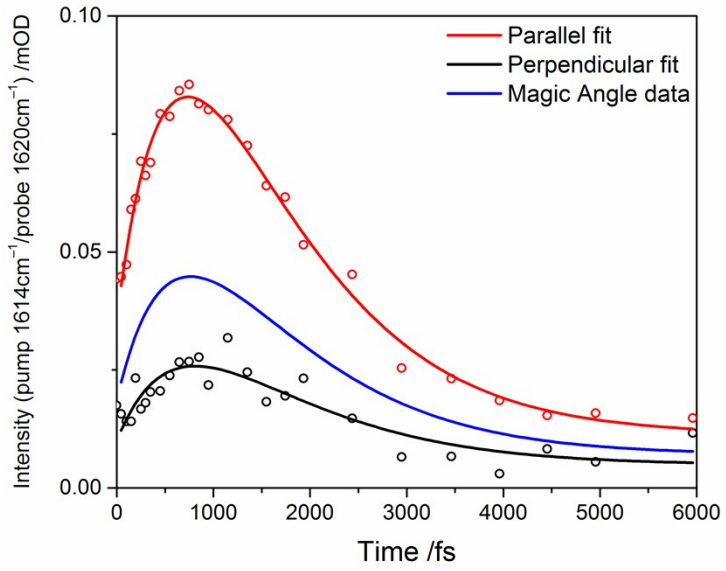
Figure SI-7



(a) Linear FTIR and (b) broadband nonlinear spectrum of α-elastin in PBS-D_2O measured at 25°C (black line) and 60°C (red line).

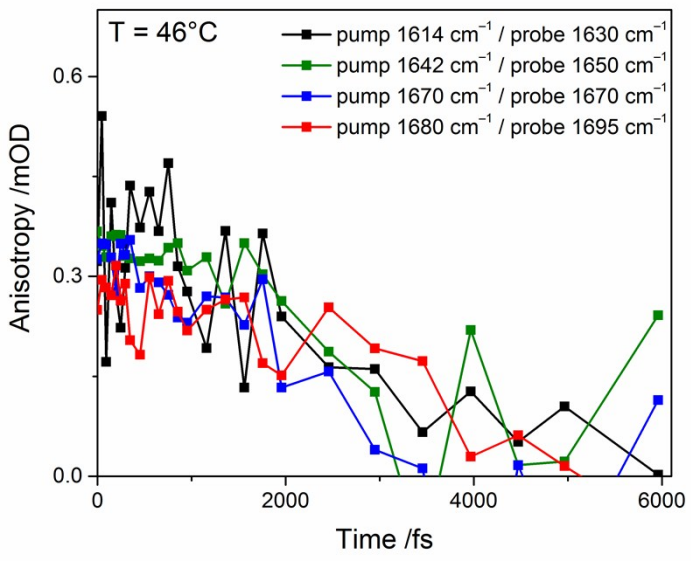
An increase of temperature causes a decrease of intensity of the A-I peak at ca. 1650 cm^{-1} in the linear spectrum (Figure SI-7a) and a slight red-shift of the ESA components and different intensity in some of the bleaching components in the transient spectrum (Figure SI-7b).

Figure SI-8



Parallel and perpendicular polarized data from pump-probe kinetics derived from narrowband nonlinear IR measurements of α-elastin in PBS-D_2O at 25°C. Pump and probe were set to 1614 and 1620 cm^{-1}, respectively. Red and black line: results of fit analysis using an exponential fit function. Blue line: magic angle profile obtained from eq. 1.

Figure SI-9



Anisotropy decays derived from narrowband pump-probe IR measurements of α-elastin in PBS-D_2O at 46°C. Pump and probe were set to different values in correspondence of the minima in the broadband profiles. Data shows no major differences in time decay. The anisotropy time constants derived from fit analysis to an exponential decay are less than 3 ps (Table 1).

References

1. S. M. Partridge, H. F. Davis and G. S. Adair, *Biochem. J.*, 1955, **61**, 11-21.